IV. REMARKS

Claims Status

Claims 1-11 were pending. Claims 12-14 are added; claims 10 and 11 are cancelled.

Objection to Specification

The specification is objected to because the first page of specification, in its present form does not properly cite the application priority data.

The priority data has been added thus obviating this ground for objection.

Claim Rejections - 35 U.S.C § 112

Claims 1-11 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention in that:

1. the recitation of phrase "gamma-sterilizable" in Claims 1-11 renders those claims vague, unclear and indefinite, because said term indicates a futuristic event because the metes and bounds for said term are not defined in the claim language.

2. the term "including" renders Claim 1 vague and indefinite because it is not clear whether said term is open, or whether the term excludes other ingredients.

3. the recitation of the phrase "hydrogen peroxide

bearing situation" renders claims 1 and 10-11 vague, unclear and indefinite, because the metes and bounds for said term are not defined in the claim language.

4. the recitation of the phrase "microbial content test agar" renders claim 8 vague, unclear and indefinite, because the metes and bounds for said term are not defined in the claim language.

5. all other claims depend directly from the rejected claims (e.g., Claim 1) and are, therefore, also stand rejected under 35 U.S.C. §112, second paragraph for the reasons set forth above.

Applicant traverses the rejection as to items 1, 3 and 4 and has amended claim 1 to obviate the rejection under item 2.

With regard to item 1, gamma sterilization is a well known art understood term. Many references exist utilizing the term, including US 6,908745, cited by the examiner, and the two articles submitted with this response. As typically utilized by those in the art, the term "gamma-sterilisable" relates to a normal radiation dose of 16 to 25 kgray, with which 10^6 Bacillus pumilus could be killed. (see page 4, lines 7-11 of applicant's specification).

With regard to item 3, the phrase "hydrogen peroxide bearing situation" means an environment in which hydrogen peroxide is present. The formulation according to the present invention neutralizes 5,000 to 10,000 ppm of

hydrogen peroxide.

In the pharmaceutical industry isolation (sterile) areas are often required. The isolation areas are typically sterilized with 35% hydrogen peroxide gas and then ventilated. Thus, the complete isolation area becomes sterile.

The remaining hydrogen peroxide concentration in the air of the isolation room is about 1 to 2 ppm. In the process of collecting 1000 L samples of air within the room for sterility testing, the hydrogen peroxide in the sample accumulates in the agar. Since hydrogen peroxide gas is soluble in water, more than 10 mL agar results in a high accumulation rate. In general, 100 to 1000 ppm of hydrogen peroxide can deposit on the agar surface. A concentration of as little as 10 ppm will kill all micro-organisms and spores. Thus micro-organisms not killed by gassing with hydrogen peroxide cannot be detected in situ without neutralizing the hydrogen peroxide.

It is this problem that applicant recognized and that applicant's self-neutralizing compositions solve.

With regard to item 4, the microbial Content Test Agar is a standard product by Difco since 1927. The enclosed Difco manual discloses the composition thereof (cf. pages 313/314).

Claim Rejections - 35 U.S.C. §103

Claims 1-11 stand rejected under 35 U.S.C. § 103 (a) as

obvious over combined teachings from Kaiser (U.S. Patent 5,968,807) in view of Atlas et al. (Handbook of Microbiological Media, CRC Press, Boca Raton, 1997, Pages 209-210 and 1272 for e.g.) and Horn (U.S. Patent 6, 908,745).

The examiner states that Kaiser teaches a culture medium comprising pancreatic digest of casein, soytone, i.e., soybean peptone and a pH indicator as well as phosphate buffer; that Horn teaches a gamma sterilizable culture medium for selective enumeration microorganisms, i.e., yeasts and fungi, and that Atlas et al. teach a variety of culture media for isolating a whole sleuth of microorganisms, especially bacteria grown in the presence of a number of chemicals.

The examiner concludes that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify teachings from Kaiser with those from Horn and Atlas et al. to obtain a culture medium comprising casein, soy peptone agar supplemented with a pH indicator, e.g., bromocresol purple and another component, e.g., sodium thioglycolate because Kaiser teaches a basic recipe for cultivating microorganisms from an environmental sample, wherein said recipe comprises casein soy bean peptone (i.e., soy tone) aga., Horn teaches a culture medium for enumerating yeast and fungi in air samples wherein said medium is sterilized with gamma radiation and Atlas et al. teach a number of recipes for microbiological culture media to cultivate microorganisms in a variety of environmental /cultivation atmospheres.

Applicants traverse this ground for rejection.

All of applicant's claims recite a hydrogen peroxide neutralizing gamma sterilizable culture medium for the detection of microorganisms in a hydrogen peroxide bearing situation comprising casein soy peptone agar.

None of the cited references, alone or taken in combination, disclose a hydrogen peroxide neutralizing culture medium for the detection of microorganisms in a hydrogen peroxide bearing situation comprising casein soy peptone agar.

Thus, this is not a situation where it may or may not be obvious to combine the references because even a combination of the references would not yield applicant's culture medium.

The problem addressed and solved by applicant, but not recognized or addressed by any combination of the references is that of determining the presence of contamination in a hydrogen peroxide containing environment (hydrogen peroxide bearing situation). This is extensively discussed on pages 1, line 8 through page 3, line 2.

With regard to claim 7, none of the references recognize the benefit of buffering with 20 to 50% MOPS with the remained phosphate buffer. (see page 4, lines 1-6 of applicant's specification)

With regard to claim 2, the surprising advantages of the

specific range of pyruvate claimed is set forth on page 4, lines 16-20. One having ordinary skill in the art at the time of the claimed invention would not have expected the pyruvate to be effective solely in this range.

Starting from the teaching of Kaiser, Horn, and Atlas et al., all of which lack the disclosure of neutralizing hydrogen peroxide directly in the agar, a person skilled in the art was not provided with the slightest indication that the hydrogen peroxide could be neutralized by the agar itself without further treatment. This possibility is directly contrary to the disclosure or suggestion of the references since, according to the cited documents, hydrogen peroxide was neutralized by bisulfite. However, bisulfite solution is not stable upon heating and treating the bisulfite by autoclaving.

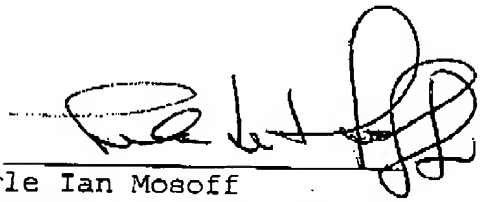
Therefore, it was not obvious for a skilled person that self-neutralization of hydrogen peroxide will be possible by using the agar according to the present invention.

Conclusion

Applicant believes that the amendments to the claims and the discussion set forth above obviate the grounds for the examiners rejection of the claims and therefore respectfully request favorable reconsideration by the examiner and early allowance of the claims. The Commissioner is hereby authorized to charge payment for any fees associated with this communication or credit any over payment to Deposit Account No. 14-1263.

Respectfully submitted,

NORRIS McLAUGHLIN & MARCUS, P.A.



By _____
Serle Ian Mosoff
Attorney for Applicant(s)
Reg. No. 25,900

875 Third Avenue - 18th Floor
New York, New York 10022
Phone: (212) 808-0700
Fax: (212) 808-0844

Customer No.: 21001

Applications

Sterilization Methods & Considerations

CYRO TECH BRIEF

This brief gives advice for:

- **Sterilization Methods**
- **Suitable CYRO Materials**
- **Effect of Sterilization on Properties of CYRO Materials**

Sterilization is defined as the total absence of living organisms. The technology required is used in industries as diverse as food processing and space exploration. Generally though, it is most often associated with healthcare. Devices that are required to be sterile are those intended to breach the body's defense mechanism, to come into contact with damaged tissue or to be implanted into the body. Most industries that use sterilization technology are regulated by a federal agency. Medical devices are regulated by the FDA. This brief will cover the most common sterilization methods.

Use of CYRO Materials in Medical Applications

CYRO offers materials that may be sterilized using Ethylene Oxide (EtO), gamma radiation, and electron beam (E-beam) radiation. These materials include: transparent CYROLITE® G-20 acrylic-based multipolymer compounds, gamma stable CYROLITE® GS-90 and CG-97 compounds, and opaque, high impact CYREX® acrylic-polycarbonate alloy.

Key physical properties of these materials have been extensively evaluated after typical gamma, E-beam and EtO sterilization.

Under these conditions, the previously mentioned materials suffer little or no physical property deterioration as shown by the change in such key properties as elongation at break and notched Izod impact.

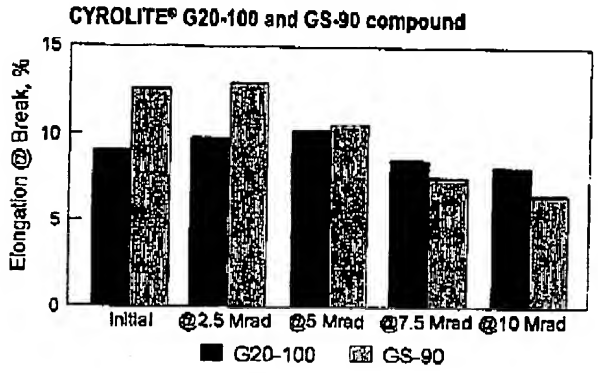
All CYROLITE compound grades maintain physical properties after exposure. However, CYROLITE G-20 compound does show some yellowing immediately after gamma and E-beam irradiation which is reduced with time.

CYROLITE GS-90 and CG-97 compounds have been developed as gamma stable grades. They exhibit minimal yellowing due to irradiation.

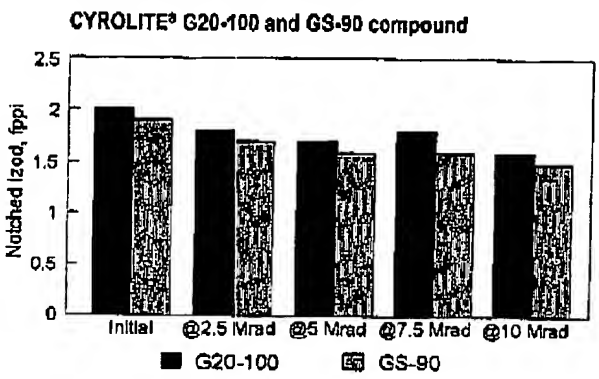
Some of the key results obtained are presented in the graphs on the following page.

Key physical properties after typical gamma, E-beam and EtO sterilization — continued from previous page

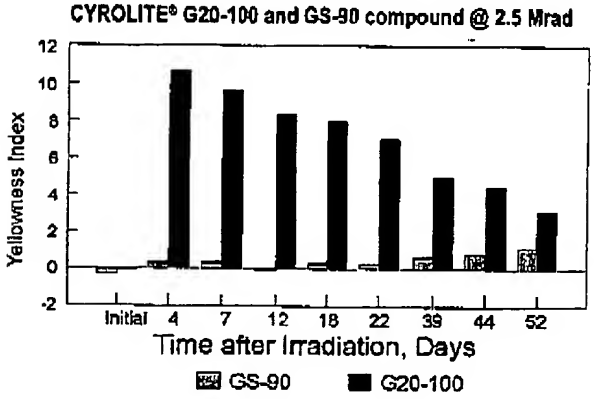
1 **Effect of Gamma Irradiation on Elongation**



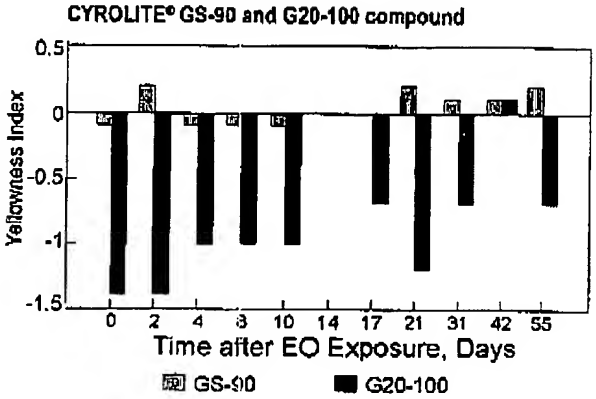
2 **Effect of Gamma Irradiation on Impact**



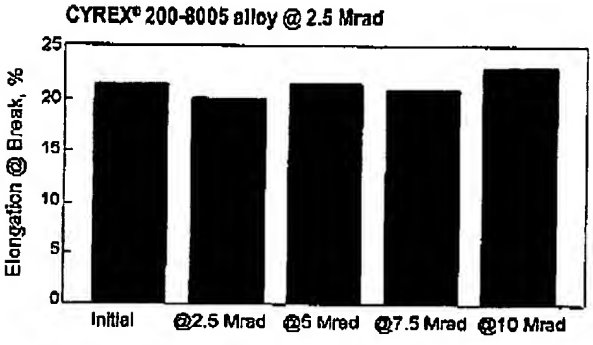
3a **Yellowness Index vs Time after Gamma Irradiation**



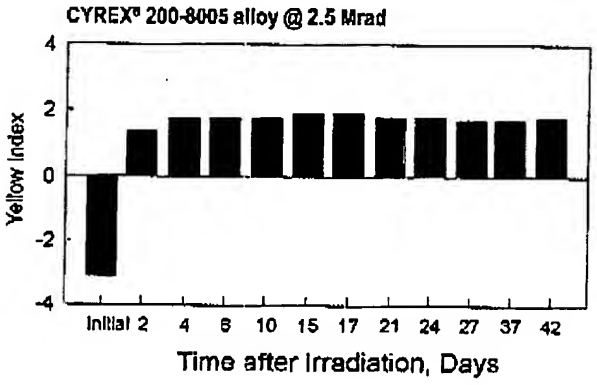
3b **Effect of EtO on Y.I.**



4 **Effect of Gamma Irradiation on Elongation**



5 **Effect of Gamma Irradiation on Y.I.**



The following recommendations and conclusions can be drawn from the information presented in the graphs on the left.

1. CYROLITE G20, GS-90, and CG-97 compounds as well as CYREX alloys show no significant loss in physical properties at an exposure level of 5 megarads.
2. CYROLITE GS-90 compound is recommended for applications requiring minimal yellowing and a high transparency level.
3. CYROLITE CG-97 compound is recommended for applications requiring minimal yellowing and superior lipid and isopropyl chemical resistance (data not shown).
4. EtO sterilization results in little to no color shift in CYROLITE compounds.
5. CYREX 200-8005 alloy is recommended for applications where transparency is not required but high impact and color retention are critical.
6. The use of EtO sterilization results in no significant property deterioration or yellowing in CYROLITE compounds or CYREX alloys.

Ionizing Radiation Sterilization

Ionizing radiation sterilization is a type of "cold" sterilization. It can employ either electron accelerators (E-beam sterilization) or radioisotopes (gamma sterilization). Electrons have relatively low penetration ability, and the use of accelerators requires careful control. Gamma-radiation sterilization usually employs ^{60}Co as the radioisotope source. A wide range of packaging materials can be used because gamma rays possess considerable penetrating ability. A dose of 2.5×10^4 Gy (2.5 megarad) is generally selected for many articles although higher levels are occasionally used.

Both E-beam and gamma sterilization effectively kill microorganisms because of their ability to break the chemical bonds of organic compounds, producing highly reactive species known as free radicals.

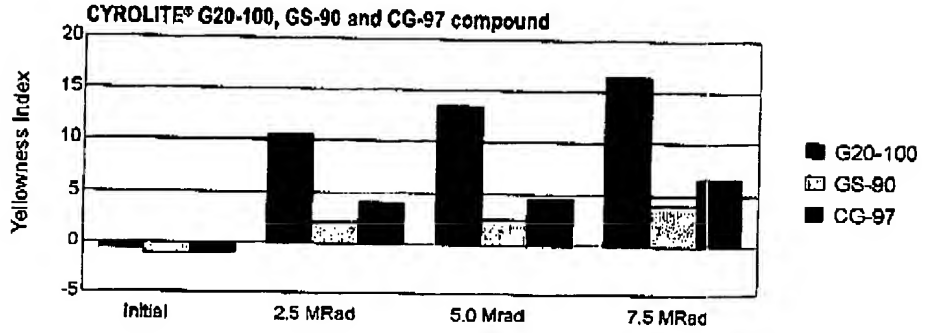
When polymers are used as the packaging or dispensing materials, the above ionizing radiation can result in chain scission which reduces the strength-related properties of the material, cross-linking which results in a stiffer but more brittle material, and color formation (yellowness) due to trapped free radicals. The highest product temperatures reached in gamma sterilization are usually in the range of 30-40°C.

This sterilization method has been extensively evaluated with the CYRO products listed above and is also recommended.

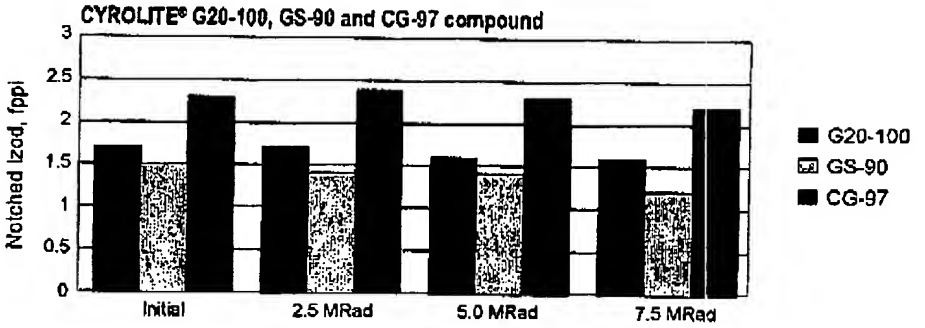
Some of the key results obtained are presented in the graphs on the following page.

Ionizing radiation sterilization -- continued from previous page

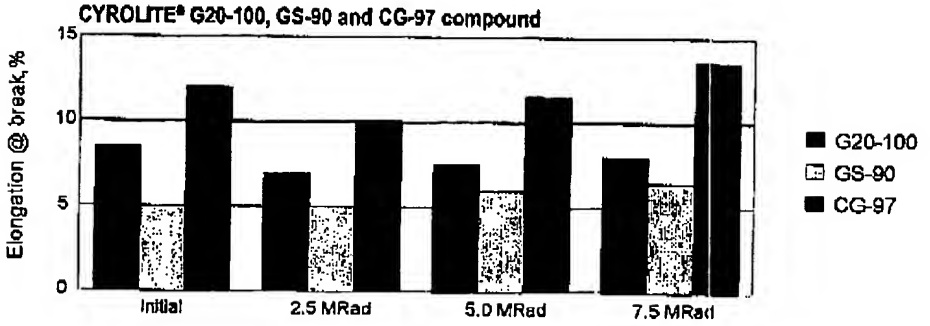
Effect of E-Beam onYellowness Index



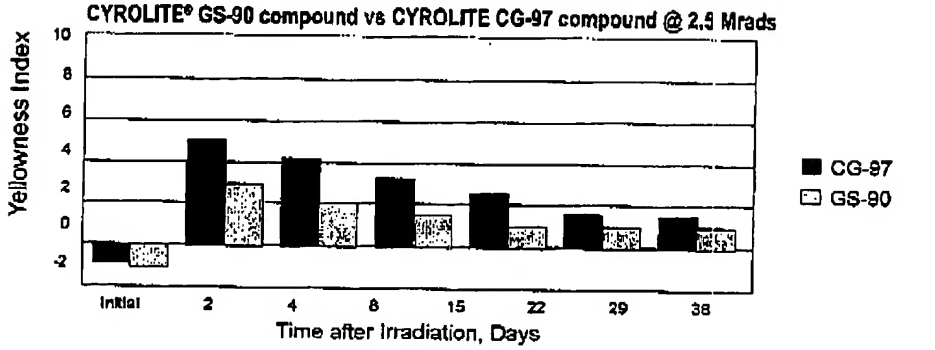
Effect of E-Beam on Impact



Effect of E-Beam on Elongation



Effect of Gamma Irradiation onYellowness Index



Gas Sterilization (EtO)

Materials that cannot withstand the temperatures and moisture of steam sterilization can use gas sterilization as an alternative. Gaseous sterilants can function at relatively low temperatures but need to be safe during handling. Another requirement is that the absorbed gas, if any, should volatilize relatively quickly. Ethylene oxide (EtO) satisfies these requirements and is the most frequent choice. Since it is highly flammable, it must be used in a carefully controlled manner, and is dispensed from a single-use cartridge or diluted with inert gases until no longer flammable. The most frequently used diluents are fluoro-carbon gases or carbon dioxide. The critical parameters are temperature, time, gas concentration, and relative humidity. Temperatures reached in ethylene oxide (EtO) gas sterilization are usually in the 50-60°C range. This method can be used with the CYRO products listed above without significant property deterioration or change in appearance.

Dry-Heat Sterilization

Dry-heat sterilization is generally conducted at 160-170°C for a minimum of two hours. Specific exposures are dictated by the bioburden concentration and the temperature tolerance of the products. Appropriate conditions must be determined throughout the material being sterilized. The equipment used is forced-air type ovens with temperature-recording. This type of sterilization is not recommended for use with CYRO materials because of the high temperatures required.

Steam-Sterilization

Steam-sterilization indicates sterilization by moist heat. The process is carried out in autoclaves using saturated steam.

Temperatures range from 115°C to 121°C and higher. Critical parameters are temperature, time, air elimination, steam quality, and absence of superheating. There must be direct steam contact which can be prevented by the presence of air; its absence is therefore considered an absolute requirement. The selection of an appropriate steam-sterilization cycle must be made after careful study of the nature of the articles being sterilized, the type and number of organisms present, type and size of each package, type of packaging material used, and other factors which may influence the performance in the application.

This method is not recommended for use with CYRO products because of the high temperatures involved.

For more information or specific questions about your project, contact a CYRO Technical Service Representative, or contact the nearest CYRO Sales Office.

Technical Center 25 Executive Boulevard, Orange, CT 06477 203-795-6081 Fax: 203-795-5800

Sales Offices

United States 100 Enterprise Dr., PO Box 5055, Rockaway, NJ 07866 973-442-6123

International 100 Enterprise Dr., PO Box 5055, Rockaway, NJ 07866 Fax: 973-442-6033

CYRO Canada Inc. 6285 Northam Drive, Unit 100, Mississauga, Ontario L4V 1X5 905-677-1388 (In Canada) 800-268-4743

Headquarters 100 Enterprise Drive, PO Box 5055, Rockaway, NJ 07866 973-442-6000

Website: http://www.cyro.com

CYRO Industries, Rockaway, New Jersey 07866

2024-0300-5RA

Sterilization

Strategies for Gamma Sterilization of Pharmaceuticals

by Ruth Garcia, Betty Howard, Rose [illegible], Judy Walker
Steris Isomedix Services (Mentor, OH)

Sterility is desirable not only for [illegible] parenterals or injectable [illegible] in drugs and [illegible]

A crucial step in pharmaceutical production is sterilization. There are many sterilization methods to choose from, such as steam, sterile filtration, ethylene oxide gas (EtO), electron beam (E-beam), and gamma radiation. Each technique has aspects that make it suitable or unsuitable for the sterilization of a particular product.

For example, EtO, while being a highly effective method, leaves behind potentially hazardous residuals and cannot reach products in airtight packages. E-beam, while being one of the fastest methods of sterilization, cannot penetrate well into dense product or bulk packaging of some products. In addition, the product complexities of heterogeneous components often require extensive product qualification. Gamma radiation can cause certain product and package materials to degrade.

GAMMA BENEFITS

Gamma radiation does have some significant advantages over other methods of producing sterile product. These benefits include:

- Better assurance of product sterility than filtration and aseptic processing.
- No residue like EtO leaves behind.
- More penetrating than E-beam.
- Low-temperature process.
- Simple validation process.

The first aspect to consider when sterilizing with gamma is product tolerance to the radiation. During use of this type of radiation, high-energy photons bombard the product, causing electron displacement within. These reactions, in turn, generate free radicals, which aid in breaking chemical bonds. Disrupting microbial DNA renders any organisms that survive the process nonviable or unable to reproduce.

However, these high-energy reactions also have the potential to disrupt bonds within the pharmaceutical formulation, to weaken the strength of packaging materials, and to cause changes in color or odor in some materials. For these reasons, drug manufacturers should perform prequalification Dmax (maximum dose) testing, whereby the drug and its packaging are subjected to a high dose of gamma radiation and then evaluated for stability and functionality.

Usually, the manufacturer will be the party responsible for drug testing. Parameters to characterize typically [illegible] stability, biocompatibility, and chemical acceptability. Per guidelines under the International Conference on Harmonization (ICH), known as Technical Requirements for Registration of Pharmaceuticals for Human Use, it is recommended to use high-performance liquid chromatography (HPLC), mass spectrometry, or gas chromatography to characterize and compare different analytical aspects of irradiated product versus nonirradiated product.

A qualified laboratory should perform package testing. It is often recommended to have an aerosol challenge performed on the product and packaging. This test entails placing the packaged product inside an aerosol chamber and exposing it to high levels of bacterial spores. The product is then subjected to a sterility test, which shows whether or not the packaging maintains a sufficient barrier.

In addition, at least one physical challenge should be performed on the packaging, if applicable. These include the peel test to determine the amount of pressure needed to open the seal; the burst test to determine the amount of pressure needed to burst the package and to locate areas of weakness in the package; and the dye

PHOTO COURTESY STERIS ISOMEDIX SERVICES (MENTOR, OH)

migration test, which determines whether dye travels through the seals of the package. If a shelf-life claim is desired, most labs will perform accelerated aging. Typically, incubation at 55°C for 6.5 weeks equals one year on a shelf (this may vary depending on the drug formulation). These tests are performed on aged products.

Performing a fraction of or all of these tests following a high dose of gamma radiation will give the manufacturer a good idea of product and packaging suitability for gamma radiation. (A high dose is usually considered to be in the 50–60-kGy range or higher, preferably twice the minimum.) Many materials are highly resistant to radiation. If possible, the manufacturer should choose materials that are resistant to the effects of gamma prior to the initial production phases.

HANDLING DEGRADATION

If a drug experiences degradation, discoloration, or any other physical malady due to the high dose of 50–60 kGy, the manufacturer can begin testing at lower doses. One method involves testing at particular intervals, such as at 5 or 10 kGy. For example, a drug that fails at 50 kGy may be stable at 40 kGy.

However, some drugs may continue to exhibit effects from the radiation at extremely low doses. Another test entails dropping the dose to half of the original high dose. This would cut the range of possible maximum doses in half. If the product is stable at the new dose, then the max dose will fall somewhere within the top half of the original high dose. If the product is still showing instability, the max dose must fall in the lower half of the original high dose tested. This method may reduce the number of irradiations necessary for establishing this information. All in all, the end product of this testing should be a solid maximum tolerated dose for the particular drug product.

Many pharmaceutical products, including parenterals and orally ingested drug products, are composed largely of water. Water dissociates as a result of exposure to radiation and is a major source of free radicals. These free radicals can cause chemical compromise, so drugs with high water content often respond poorly to irradiation.

Steris' new JS 10,000 continuous and incremental Cobalt-60 irradiator is ready to process customers' products.

Performing irradiation on product in a frozen state can mitigate these effects. If the product can be safely frozen and thawed, the potential exists to irradiate it without, or with less, product degradation. Freezing the drug traps free radicals in the ice crystals, reducing their freedom to move about. This may induce them to recombine with each other, rather then disrupt molecules in the product itself. This would possibly improve drug resistance to degradation during gamma irradiation. Other options such as freeze-drying and/or using free-radical scavengers may also alleviate the degradation effects seen in some products.

FINDING THE RIGHT DOSE

The next step is to set the minimum sterilization dose, which will provide the desired sterility assurance level (SAL). There exist two commonly used, industry accepted, validation techniques, with several variations for special circumstances. The first technique for discussion, Method 1, is found in AAMI/ANSI/ISO 11137:1994, "Sterilization of Health Care Products: Requirements for Validation and Routine Control — Radiation Sterilization."

Method 1 encompasses product with bioburden up to 1 million colony-forming units (CFUs). It allows for extremely low and high doses and is well known throughout the gamma sterilization industry. The steps are simple and straightforward. First of all, 10 product samples from each of three separate production batches must have bioburden testing performed on them. This quantitative measure, or count, of the number of organisms on the unsterilized product provides an excellent tool for determining the minimum dose necessary for sterilization.

Bioburden tests should be accompanied by a determination of recovery efficiency. This allows the laboratory to calculate a more accurate bioburden number. The average bioburden of each batch and the overall average of all product units should be determined. If any single-batch bioburden level is more than twice that of the overall bioburden, that batch average should be used. Otherwise, the overall average should be used.

Afterward, the verification or sublethal dose must be set. Using AAMI/ANSI/ISO 11137 Table B.1, find the bioburden number equal to or just higher than that of the product. Follow the row to the column labeled SAL 10^{-2}, where the verification dose will be found.

The final phase includes testing for Bacteriostasis/Fungistasis (B/F) and setting the verification dose. The B/F test validates the sterility test by determining whether the product formulation inhibits bacterial or fungal growth. If inhibition is seen, steps must be taken to neutralize it. The test is required only once in the lifetime of a product, but it is recommended annually. Without such a test, sterility-testing results are meaningless.

To begin the verification dose experiment, send 103 product units (100 for sterility testing and 3 for B/F) to the sterilization provider for irradiation at the verification dose ± 10%. If the

dose exceeds the prescribed verification dose by more than 10%, then the product must be sacrificed and new product irradiated. If the dose is lower than 90% of the prescribed dose, the remainder of the testing may be performed and a failing test would allow for a retest.

The product should then be sent to the laboratory for sterility testing and B/F testing. If two or fewer sterility tests turn positive, the product has passed the validation, and the next step is to find the sterilization dose. Manufacturers should follow the same row in Table B.1 from which the verification dose was taken, to the column marked SAL 10^{-6}. This is the minimum sterilization dose. The product now qualifies to be irradiated at a range from the minimum dose to the maximum dose determined during the high-dose materials testing.

The second type of validation is commonly known as VDmax. Found in AAMI TIR 27:2001, "Radiation Sterilization, Substantiation of 25 kGy," this method requires fewer products and results in a minimum sterilization dose of 25 kGy. However, only products with 1000 CFU or less qualify.

The first step of this process is identical to that of Method 1. Bioburden data from 10 products from each of three separate production batches should be collected. Using Table 2 of the TIR, the bioburden number equal to or just greater than the product's average bioburden is found. The sublethal dose is found by following the row to the column labeled "Verification dose" (SAL 10^{-1}). Send 13 product units (10 for sterility testing and 3 for B/F) to the sterilizer for irradiation at the verification dose ± 10%. Once the irradiation is complete, send the products to the laboratory for sterility testing. If one or fewer sterility tests turn positive, the product can be irradiated at a minimum dose of 25 kGy. If two positive sterility tests occur, a retest should be performed on 10 additional products. This time, no positives are allowed for substantiation of 25 kGy. Should positives occur, another dose-setting method must be used.

Also contained in AAMI 11137 is an alternative validation procedure referred to as Method 2. Method 2 provides for dose setting based on the actual radiation resistance of microorganisms as they naturally occur on a product. Of the methods cited, it can provide the lowest possible minimum dose. It is not used as frequently as Method 1 or VDmax, due to more sample requirements and associated costs.

Method 2 uses incremental dose data to select a verification dose. Groups of samples from three production batches are irradiated in dose increments up to the point where an SAL of 10^{-2} can be determined. A Method 2 validation starts with the random selection of 280 samples (Method 2A) or 260 samples (Method 2B) from each of three production batches of product. Samples are then designated in groups of 20 samples for each dose increment. Method 2A uses nine increments in 2-kGy increments, and 2B uses eight doses at 1-kGy increments. All samples are tested for sterility. After the results of sterility tests are known, a series of calculations described in AAMI 11137 (section B3.4.2) a verification dose (D*kGy) is determined.

An additional 100 samples from the batch designated from the initial sterility tests are irradiated at the verification dose and tested to confirm sterility. Following these sterility tests, a sterilization dose is calculated using the equation appropriate to the specific method chosen (2A or 2B).

In extreme circumstances in which all efforts to neutralize bacteriostatic agents have been exhausted and other sterilization methods are unsuitable, dose setting can be done with inoculation of the product. The practice of inoculation, commonly used in the past, is not currently recommended unless it is impossible to collect natural bioburden data from the product. Fortunately, in most cases, product inoculation is not necessary.

The organism most commonly used for radiation challenge is *Bacillus pumilis*. It was once believed that this organism was highly resistant to gamma. However, many organisms naturally occurring in medical products are more resistant to radiation than *B. pumilis*, rendering this a poor surrogate organism. If no alternative exists, however, this method may be acceptable. A D10 value (D value) of an organism, in this case, is the amount of radiation (quantity of kGy) necessary to reduce the bioburden level by 1 log.

An example of a published D value for *B. pumilis* is 1.7 kGy. Some caution should be taken in using a published D value, as D values can vary depending on the technique used to determine them and/or the inoculation substrate. Also, D values, or the resistance of an organism to gamma radiation, can change over time, analogous to antibiotic resistance in microorganisms. However, if this is the method to be used, the following is an example of the calculation for determining minimum sterilization dose.

Inoculation with 10^6 (1,000,000 organisms):

SAL = 10^{-6}
10^6 to 10^{-6} = 12 log reduction
D value 1.7 kGy × 12 log reduction
= 20.4 kGy
20.4 kGy = 10^{-6} SAL dose

The following calculation determines the necessary verification dose for 10 products to show the efficacy of the above 20.4-kGy sterilization dose:

Log bioburden log (1/#samples)] × d-value = verification dose
Log 1,000,000 – log (1/10)] × 1.7 = verification dose
[6 – (–1)] × 1.7 = verification dose
[6 + 1] × 1.7 = verification dose
7 × 1.7 = verification dose
11.9 kGy = verification dose

A radiation dose of 11.9 kGy ± 10% is applied to 10 product units, which are then sent to a lab for sterility testing. If no more than one test out of 10 turns positive, the sterilization dose, in this example, 20.4 kGy, is validated.

Finally, whichever method is used, the manufacturer must verify the dose every 3 months in an experiment known as a Quarterly Dose Audit. To do this, 10 samples must be sent to the laboratory for bioburden testing.

Furthermore, every organism cultured during the bioburden test should be identified, at minimum with a colony morphology and gram stain. Simultaneously, repeat the original verification dose experiment for whichever method was used during the original validation. For example, if Method 1 determined the original sterilization dose, then the Method 1 verification experiment must be repeated. The original verification dose, or a dose augmented from a past dose audit, is the dose that must be used.

The quarterly bioburden samples serve as a trend-analysis tool. A new verification dose should not be determined from new bioburden data. Should a product fail a dose audit, the bioburden data may hold valuable clues as to why the failure occurred, e.g., a spike in bioburden number or shift in organism types. If neither of these is the case, there is possibly an increase in the radiation resistance of the organisms.

A dose audit failure requires a dose augmentation. The augmentation amount is found in the dose-setting table used in the original validation. Beyond all of this, the dose audit should also include manufacturing environment monitoring, such as water testing, air sampling, and contact agar plates. Although regular environmental monitoring is recommended at shorter intervals, such testing quarterly meets the minimum requirements.

The AAMI dose-setting methods described here are only recommendations and do not exclude other dose-setting procedures that may be deemed more appropriate by their users. The AAMI methods are widely accepted in North America. When properly applied, they have been accepted by regulatory groups as valid dose-setting procedures.

AAMI guidelines are regularly reviewed and updated through collaboration by industry experts (the latest drafts under consideration are 11137-01, 02, and 03, which will encompass the methods cited here in 11137:1994 and TIR 27) and are designed to provide a guideline that encompasses the latest in industry knowledge and requirements.

Each method has advantages and disadvantages, and care must be used in selecting a method that best fits the needs and limitations of the product being evaluated. These methods can provide an acceptable and straightforward means of substantiating dose selection for pharmaceutical products.

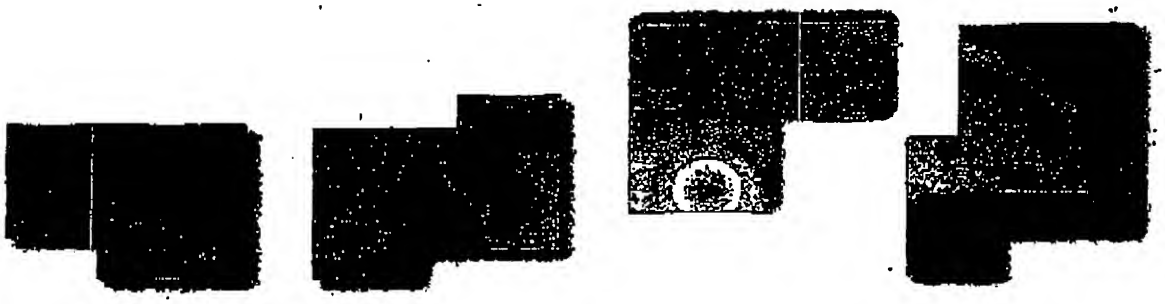
Following this guidance will aid in the successful validation of any radiation-stable pharmaceutical product for gamma radiation sterilization. The ideal time for considering the method of sterilization is at the concept stage, so that gamma-compatible materials can be chosen and the effects on product safety and efficacy can be considered. With the variety of materials currently available, many pharmaceuticals and most packaging materials can satisfactorily withstand the rigors of gamma processing.

Steris Isomedix Services provides technical support during all of these processes, including research, turnkey validations, special projects, and technical information. ■

Reprinted from *Pharmaceutical & Medical Packaging News*, May 2004 • Copyright © 2004 Canon Communications LLC

Ditto Manual

11th Edition



First Edition	1927
Second Edition	1929
Third Edition	1931
Fourth Edition	1933
Fifth Edition	1935
Sixth Edition	1939
Seventh Edition	1943
Eighth Edition	1948
Ninth Edition	1953
Reprinted	1953
Reprinted	1956
Reprinted	1958
Reprinted	1960
Reprinted	1962
Reprinted	1963
Reprinted	1964
Reprinted	1965
Reprinted	1966
Reprinted	1967
Reprinted	1969
Reprinted	1971
Reprinted	1972
Reprinted	1974
Reprinted	1977
Tenth Edition	1984
Reprinted	1985
Reprinted	1994
Reprinted	1996
Eleventh Edition	1998

Procedure

Materials Provided
Micro Assay Culture Agar
Micro Inoculum Broth

Materials Required But Not Provided
Glassware
Autoclave
Incubator
Inoculating needle
0.9% NaCl

Method of Preparation
Micro Assay Culture Agar
1. Suspend 47 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve.
3. Dispense 10 ml amounts into 16-20 mm diameter tubes.
4. Autoclave at 121°C for 15 minutes.
5. Agitate tubes prior to solidification to disperse the flocculent precipitate.

Micro Inoculum Broth
1. Dissolve 37 grams in 1 liter distilled or deionized water.
2. Dispense 10 ml amounts into tubes of 16-20 mm diameter.
3. Autoclave at 121°C for 15 minutes.

Stock Cultures
1. Prepare stock cultures in triplicate on Micro Assay Culture Agar, inoculating tubes using a straight-wire inoculating needle.
2. Incubate tubes at 30-37°C for 18-24 hours.
3. Store at 2-8°C.
4. Transfer cultures at weekly or twice-monthly intervals.

Assay Inoculum
1. Subculture from a 16-24 hour stock culture of lactobacilli in Micro Assay Culture Agar into a 10 ml tube of Micro Inoculum Broth.
2. Incubate at 35-37°C for 16-24 hours or as specified in the assay procedure.
3. Centrifuge the culture and decant the supernatant.
4. Resuspend cells in 10 ml of sterile 0.9% NaCl solution or sterile single strength basal assay medium.
5. Wash the cells by centrifuging and decanting the supernatant two additional times unless otherwise indicated.
6. Dilute the washed suspension 1:100 with sterile 0.9% single strength basal assay medium or as indicated. Where applicable, adjust inoculum concentration according to limits specified in AOAC[1] or US Pharmacopeia.[2]

Specimen Collection and Preparation
Prepare samples for assay according to references given in the specific assay procedure. Dilute assay samples to approximately the same concentration as the standard solution.

Test Procedure
For a complete discussion of vitamin assay methodology, refer to appropriate procedures.[1,2]

Results
For test results on vitamin assay procedures, refer to appropriate procedures.[1,2]

Limitations of the Procedure
1. Test organisms used in assay procedures must be cultured and maintained on media recommended for this purpose.
2. Follow assay directions exactly. The age, preparation and size of inoculum are extremely important factors in obtaining a satisfactory assay result.
3. Although other media and methods may be used successfully for maintaining cultures and preparing inocula, uniformly good results will be obtained if the methods described are followed exactly.
4. Aseptic technique should be used throughout the microbiological assay procedure.
5. The use of altered or deficient media may create mutants having different nutritional requirements. Such organisms will not produce a satisfactory test response.

References
1. Association of Official Analytical Chemists. 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
2. The United States Pharmacopeial Convention. 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc. Rockville, MD.

Packaging

Micro Assay Culture Agar	100 g	0319-15
	500 g	0319-17
Micro Inoculum Broth	500 g	0320-17

Bacto® Microbial Content Test Agar

Intended Use
Bacto Microbial Content Test Agar is recommended for the detection of microorganisms on surfaces sanitized with quaternary ammonium compounds.

Also Known as
"Tryptic Soy Agar with Lecithin and Polysorbate 80" (TSALT) and "Casein Soy Peptone Agar with Polysorbate 80 and Lecithin" are common terms for Microbial Content Test Agar. Tween 80® is also known as Polysorbate 80.

Summary and Explanation
Microbial Content Test Agar is a modification of Tryptic Soy Agar with Lecithin and Tween 80. The formulation is recommended for determining the sanitation efficiency of containers, equipment and work areas (environmental monitoring). The Lecithin and Tween in the formula inactivate some preservatives that may inhibit bacterial

growth, reducing "preservative carryover."[1] The formulation is recommended for the Acrobic Plate Count (Microbial Limit Test) for water miscible cosmetic products containing preservatives.[1]

Principles of the Procedure

Microbial Content Test Agar contains Tryptone and Soytone which provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Lecithin and Polysorbate 80 are added to neutralize surface disinfectants.[2,3,4] Lecithin is added to neutralize quaternary ammonium compounds. Polysorbate 80 is incorporated to neutralize phenols, hexachlorophene, formalin and, with lecithin, ethanol.[3] Sodium Chloride provides osmotic equilibrium. Bacto Agar has been incorporated into this medium as a solidifying agent.

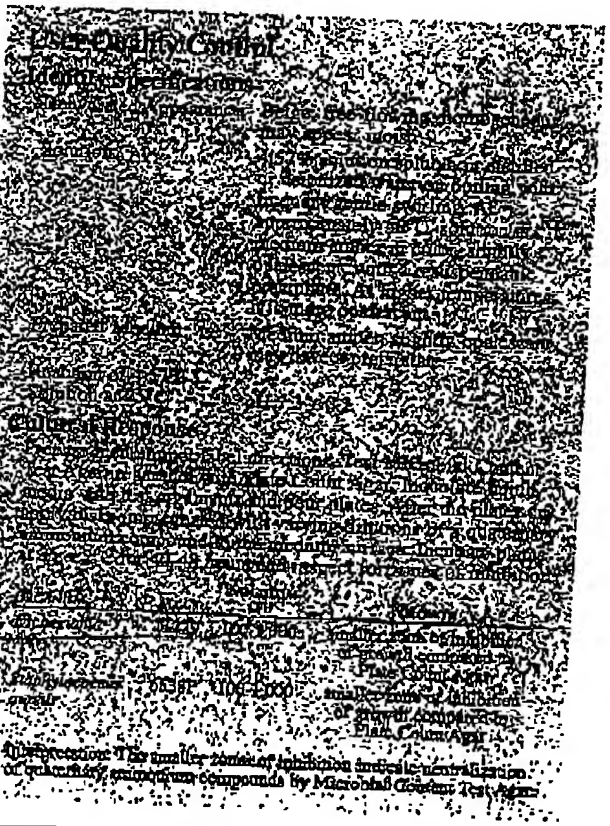
Formula

Microbial Content Test Agar

Formula Per Liter

Bacto Tryptone	15 g
Bacto Soytone	5 g
Sodium Chloride	5 g
Lecithin	0.7 g
Polysorbate 80	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C



Interpretation: The smaller zones of inhibition indicate neutralization of quaternary ammonium compounds by Microbial Content Test Agar.

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Microbial Content Test Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Sterile Petri dishes

Method of Preparation

1. Suspend 45.7 g in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely with frequent careful agitation to dissolve, 1-2 minutes.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Consult appropriate references.[4]

Test Procedures

Microbial Content Test Agar is used in a variety of procedures. Consult appropriate references for further information.[4]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Because the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on the medium.
2. The effectiveness of preservative neutralization with this medium depends on both the type and concentration of the preservative(s).

References

1. **Orth, D. S.** 1993. Handbook of Cosmetic Microbiology. Marcel Dekker, Inc., New York, NY.
2. **Quisno, R., J. W. Gilby, and M. J. Foter.** 1945. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am. J. Pharm. 118:320-323.
3. **Erlandson, A. L., Jr., and C. A. Lawrence.** 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science 118:274-276.
4. **Brummer, B.** 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. App. Environ. Microbiol. 32:80-84.